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Fingerprinting outdoor air environment using microbial volatile organic compounds (MVOCs) – A review

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ABSTRACT

The impact of bioaerosol emissions from urban, agricultural and industrial environments on local air quality is of growing policy concern. Yet the risk exposure from outdoor emissions is difficult to quantify in real-time as microbial concentration in air is low and varies depending on meteorological factors and land use types. While there is also a large number of sampling methods in use, there is yet no standardised protocol established. In this review, a critical insight into chemical fingerprint analysis of microbial volatile organic compounds (MVOC) is provided. The most suitable techniques for sampling and analysing MVOCs in outdoor environments are reviewed and the need for further studies on MVOCs from outdoor environments including background levels is highlighted. There is yet no rapid and portable technique that allows rapid detection and analysis of MVOCs on site. Further directions towards a portable GC–MS coupled with SPME or an electronic nose are discussed.

1. Introduction

Bioaerosols or the biological particles of aerosols are predominantly formed by microbial, plant and animal origin [1]. Bioaerosols are ubiquitous in the environment and due to their small particle size (<2.5 μm) are easily dispersed in the air [2]. Given the potential high concentration of bioaerosols from urban, agricultural and industrial emissions (such as composting and other biowaste processing facilities), its impact on local air quality is of a growing public health concern [3,4]. It is well known that the presence of pathogenic microorganisms in air (bioaerosols) can induce respiratory diseases and infections [5] including asthma [6,7]. Consequently, there is increased concern about the accidental or deliberate release of biological materials in the environment and the associated impacts on human and/or animal health and the economy [8] similar to the Q-fever outbreak in the Netherlands caused by the bacteria *Coxiella burnetii* [9,10]. Yet, understanding the identities, distribution and abundance of airborne microorganisms remains in its infancy and the risk of exposure to bioaerosols occupationally and to the public from urban, rural and agricultural environments is difficult to quantify in real time [4,11,12].

Current bioaerosol monitoring methods are labour intensive, time consuming, expensive and often not reproducible [13,14]. In addition, it is difficult to capture sufficient amounts of material to generate statistically distinguishable and reproducible patterns for bioaerosol identification and classification [15]. Some instruments have been specifically designed for bioaerosol collection (eg impaction onto agar plates), while many more have been adapted from other applications (eg filters, impingers). No one method is favoured over another, and the choice is typically determined by the aims of the study. For example, impaction onto agar is suitable for cultivation-based studies and offers the possibility of size fractionation. However, the stress on the bioaerosols due to impaction may reduce the retrieval of culturable microorganisms. Consequently, there may be an underestimation of the diversity and quantity of microbial load. Additionally, differences in sampling strategies can hamper data comparison. Traditional culturing methods have been used to determine the identity and concentration of bioaerosols, despite less than 1% of viable microbes being culturable under standard laboratory conditions under standard laboratory conditions [16]. Other technologies have also been used including microscopy, immunochemistry, flow cytometry, Raman spectroscopy, Fourier transform infrared spectroscopy (FT-IR),

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fluorescent aerodynamic particle sizer (FLAPS) [17]. These techniques are expensive but suitable for the identification and numeration of microbial species. More recently real-time biosensors such as Wideband Integrated Bioaerosol Sensor (WIBS) [18] and Spectral Intensity Bioaerosol Sensor (SIBS) [19] or biosensors based on microfluidic techniques [20] and carbon nanotube based biosensors [14] are getting special attention. These techniques provide real time measurements and are able to discriminate biological particles from the total particles in air.

The use of mass spectrometry (MS) has also been reported for the analysis of aerosolised material of biological origin, including the Bioaerosol Mass Spectrometer (BMAS) [21]. BMAS is based on the analysis of the mass spectrum of organism-specific peptides and proteins as a signature to differentiate microbial species [4]. Microbial phospholipid fatty acids (PLFAs) are other type of biomarkers used to provide insight into the broad composition of microbial communities as well as to identify some specific microbial taxa [4]. However PLFAs have a low taxonomic resolution and since different microorganisms can have the same PLFAs in common, microbial identification cannot be conducted at the species level [4,22].

The recent advances in analytical techniques open a new door for the chemical characterisation of bioaerosol. Specifically, chemical analysis of microbial volatile organic compounds (MVOCs) can be a reliable and rapid assessment of the nature of ambient bioaerosols [23,24] as microbial communities express different MVOCs profiles depending in which environment they are in Ref. [25]. Further to this, it has been shown that species-specific volatiles may serve as marker compounds for the selective detection of pathogenic microbial species in indoor and outdoor environments [24]. MVOCs are secondary metabolites produced by fermentation and are volatile due to their physicochemical properties (low molecular weight, low boiling point and high vapour pressure) [26]. Characterising and quantifying MVOCs, can also be used as a proxy approach to estimate microbial concentration [27]. MVOCs analysis have been applied in health care for example to diagnose Crohn's disease from urine [28], or to detect invasive *Aspergillus fumigatus* from breath samples [29]. They have been also used for the detection of explosives, warfare and drugs [30] and at composting facilities [31]. However, there is lack of studies from other environmental sources such as wastewater treatment plants, anaerobic digestion plants, agricultural farms or urban and recreational areas. Concentration of MVOCs in the environment can be highly variable between replicates and locations due to numerous factors including among others the microbial source (substrate) [32], the distance from the source, the weather conditions, wind direction and landscape topography [33]. This variability is further illustrated in Table 1, where the most common MVOCs reported for indoor and outdoor environment are summarised. Overall MVOCs concentrations tend to be higher in indoor environments mainly because these environments remain closed and ventilation rates are often low compared to outdoors [34]. MVOCs concentrations can also vary significantly; for example dimethyl-disulfide concentration range between 16 and 263,000 ng m^{-3}. Although some pathogen microorganisms produce specific MVOCs which are indicative of microbial contamination it is still to date difficult to discriminate a microbiologically contaminated area from a non-contaminated area [35]. This is due to the lack of MVOCs data available for different environments and especially outdoor environments. Until now, most of the studies are focused on studying MVOCs from indoor built environments while there are limited studies conducted on the outdoor environments, mainly focusing on biowaste and animal farm facilities. There is also a lack of information on how to determine what actually constitutes a MVOC contaminant concentration threshold and therefore the concentration limits reported in the literature are often contradictory. Moreover, there is not yet an established database of standard/reference MVOCs for specific environments (i.e. rural *vs* agricultural *vs* industrial *vs* urban) [36] and researchers measure different MVOCs which makes comparison between studies impossible. For example, Lorenz et al. [37] collected indoor air from buildings over a 240 min period at 0.5 ml min^{-1} flow rate with SKC pumps and TD tubes coated with activated charcoal (Anasorb®). They identified 1-octen-3-ol, dimethyl disulphide and 3-methylfuran as the main indicators of microbial growth and determined that there is an indoor microbial contamination source when the detection of one of these MVOCs is present at concentrations above 50 ng m^{-3} (see SI Table 1). They also reported that a microbial contamination source could be considered when the sum of eight MVOCs including 1-octen-3-ol, 3-methylfurane, dimethyldisulfide, 3-methyl-1-butanol, 2-pentanol, 2-hexanone, 2-heptanone, 3-octanone together with at least one of the main MVOCs indicators of microbial growth equals or exceeds 500 ng m^{-3}. In contrast, Korpi et al. [44] indicated, based on published data of indoor studies from buildings, that microbial air contamination of an environment can be identified when the concentrations of specific MVOCs reach or exceed the threshold values reported in SI Table 1. Thus, human risk of exposure to MVOCs is still difficult to quantify in real time and this risk is directly linked to the concentration and type of microorganisms [39–41]. Nevertheless, a more in-depth understanding of MVOCs is still required and performance of specific analytics remains to be established to allow separation and detection of microbial molecular signatures. Specifically the detection, identification and classification of MVOCs can offer insights to microbial activity, abundance, community structure, community-level and physiological activity expressing characteristic profiles through distinct chromatograms. Moreover, untargeted metabolomics offers broader exploration of metabolites with opportunities to identifying new compounds. In particular, species-specific MVOCs have a potential to be used as marker for the selective detection of fungal and bacterial species in the environment.

The purpose of this paper is to review the current state of art on sampling and analysis of MVOCs from urban, rural and agricultural emissions. We highlight the challenges on sampling, analytical determination and speciation, before proposing a way forward to develop rapid, sensitive and reproducible tools which allow the characterisation of bioaerosols by focusing on MVOC fingerprints analysis from a range of environmental sources.

2. Sample collection and analysis

Currently, there is a lack of standard guidelines for sample collection and analysis of MVOCs. Albrecht et al. [41] published a set of recommendations of how to design studies and which sampling strategies to use for airborne microorganisms, MVOC and odours in the surroundings of composting facilities but there is no information available for sampling in other environments. Methodologies presented in published studies are very variable, ranging in sampling time from a few minutes to up to 48 h with different flow rates and using a variety of different devices. For all of these reasons, the comparison of data from different methods in published studies is challenging. The sampling locations depend on the wind direction on the day of sampling. For example the highest bioaerosol concentrations are found downwind of the composting activities [46]. In fact, the analytical method used will affect the obtained VOC profile. A schematic representation of the main available techniques with GC–MS as posterior analysis is detailed in Fig. 1. Briefly, MVOCs are often collected using thermal desorption (TD) tubes, impingers or filters. The main advantage of TD tubes is that the air samples do not require sample preparation for

Table 1
Concentrations of most common MVOCs (ng m^{-3}) in indoor and outdoor environments.

Compound	Indoor environments				Outdoor environments
	Living environments[a,e]	Problem buildings[b]	Normal buildings[c]	Broiler sheds[d]	Compost facilities[e]
2-Methyl-1-propanol	3000–10,400	nd-1740	340–1380	na	na
2-Methyl-1-butanol	na	na	na	na	170–1400
3-Methyl-1-butanol	3000	175–260,000	8700–110,000	nd-25,000	300–35,000
3-Methyl-2-butanol	3610	190–1190	nd-160	na	nd-70
3-Octanol	5330–8800	nd-8860	nd-40	na	nd-140
1-Octen-3-ol	5240–11,800	nd-904,000	nd-7000	300–6000	nd-1900
2-Octen-1-ol	5240–21,500	1560–266,000	nd-14,000	na	nd-6820
2-Pentanol	3610–4800	nd-1400	1700	na	na
2-Methylfuran	6300	na	na	na	75–1500
3-Methylfuran	3360	nd-1800	nd-160	na	nd-110
2-Penthylfuran	5100	na	na	na	85–1240
2-Heptanone	4670–16,900	nd-97	nd-1200	na	nd-3000
2-Hexanone	4100	25–8800	7–2900	na	nd-800
3-Octanone	5240–11,600	nd-3020	nd-3000	na	nd-2000
Geosmin	6000–7460	nd-550	nd-50	na	nd-10
Borneol	6900	na	na	na	160–7000
2-Methyli-sorbenol	6880	nd-2800	nd-560	na	nd-1180
Dimethylsulfide	1700	na	na	nd-1700	<50–3300
Dimethyl disulfide	3850–263,000	16–90	nd-710	nd-263,000	nd-6000
2-Isopropyl-3-methoxy-pyrazine	6220	nd-9500	nd-3	na	nd-340

na = not analysed; nd = not detected.
[a] Living environments = houses [35,43,44].
[b] Problem buildings = Buildings with damp problems [34,38].
[c] Normal buildings = without damp problems or non-complaint areas [35,44,45].
[d] Broiler sheds [44].
[e] Compost facilities [39,45].

gas chromatography coupled to mass spectrometry (GC–MS) analysis whereas air samples collected using filters or Coriolis devices need extraction steps. A range of statistical analysis such as principal component analysis (PCA), canonical correspondence analysis (CCA) or hierarchical cluster analysis (HCA) or multidimensional scale analysis (MDS) can be then carried out to identify correlations, trends and specific MVOC markers between different outdoor environments. A summary of the most commonly used sampling and analytical techniques for MVOCs studies and their advantages and disadvantages of each one of them is also provided in Table 2.

2.1. Sampling techniques

MVOCs are very diverse in polarity and chemical structure and they are present in trace levels; therefore efficient sampling methods are required. A range of sampling techniques is available to characterise ambient MVOCs (Table 2). With reference to identification and characterisation of MVOCs, sampling is carried out by charcoal pads [47], thermal desorption tubes (TD) [48,49] or by ion mobility spectrometry (IMS) gas sensors [50] including the electronic nose [40]. Chemical analysis is often done by GC–MS as it offers a good chromatographic separation of the compounds and high sensitivity [51].

2.1.1. Activated charcoal pads

Charcoal pads are diffusive samplers and are cheap, light and easy to use since they can be operated without electricity [52]. However, there are few studies reporting MVOCs data using charcoal pads. This technique is advantageous when monitoring an area during hours, days or weeks by static diffusive passive sampling [47,52]. Sample preparation is easy as charcoal pads just need to be solvent extracted with carbon disulphide [47]. This is also a limitation, since there is a probability of losing sample and, in fact, this technique is not very sensitive.

2.1.2. Impingers

Glass impingers and cyclones collect air samples into a liquid medium by suction. These techniques have a potential for MVOCs analysis as allow the collection of high sample volume in a short time period (10–30 min) without microbial loss caused by drying issues as the microorganisms are collected within a liquid [53]. The disadvantage of the impingers is the potential loss of the collection fluid due to the high flow rate used. For field work cyclone techniques are preferable as they have less liquid loss for evaporation compared to glass impingers (15% loss *vs* 30% during 30 min sampling time), are easier to sterilise and also easier to transport [54]. The short sampling time of impingers compared to longer sampling techniques such as filtration or charcoal pads can affect the reproducibility, obtaining more variability in concentrations between replicates. Currently, a variety of high-volume samplers have been used for bioaerosol monitoring, including the BioGuardian air sampler (InnovaTek, Inc.), the SpinCon air sampler (Specter Industries, Inc.) or the BioCapture 650 (MesoSystems Technology, Inc.) Han [55]. The cyclone sampler Coriolis®μ is a further high volume sampling unit [1,56] but differing from the rest of the impingers, its size makes it easy to handle and it is easily portable [57]. “BioSampler” (SKC, Eight Four,PA, USA) is an all-glass cheap impinger (Ace Glass Inc., N.J., USA), but less efficient than the other mentioned techniques change [17].

2.1.3. Thermal desorption tubes

Among all the sampling techniques used for environmental MVOCs, thermal desorption (TD) tubes filled with Tenax® or Tenax®-Carbotrap 50/50 v/v are the most common ones [58]. Gallego et al. [59] compared between TD tubes coated with a Tenax® or a multi-sorbent bed of carbonaceous adsorbents and they recommended the use of the latter to assure a complete gathering of very volatile organic compounds without losing any sample. TD tubes coated with activated charcoal (Anasorb®) allow more sampling time and are good for collecting very volatile MVOCs but, for

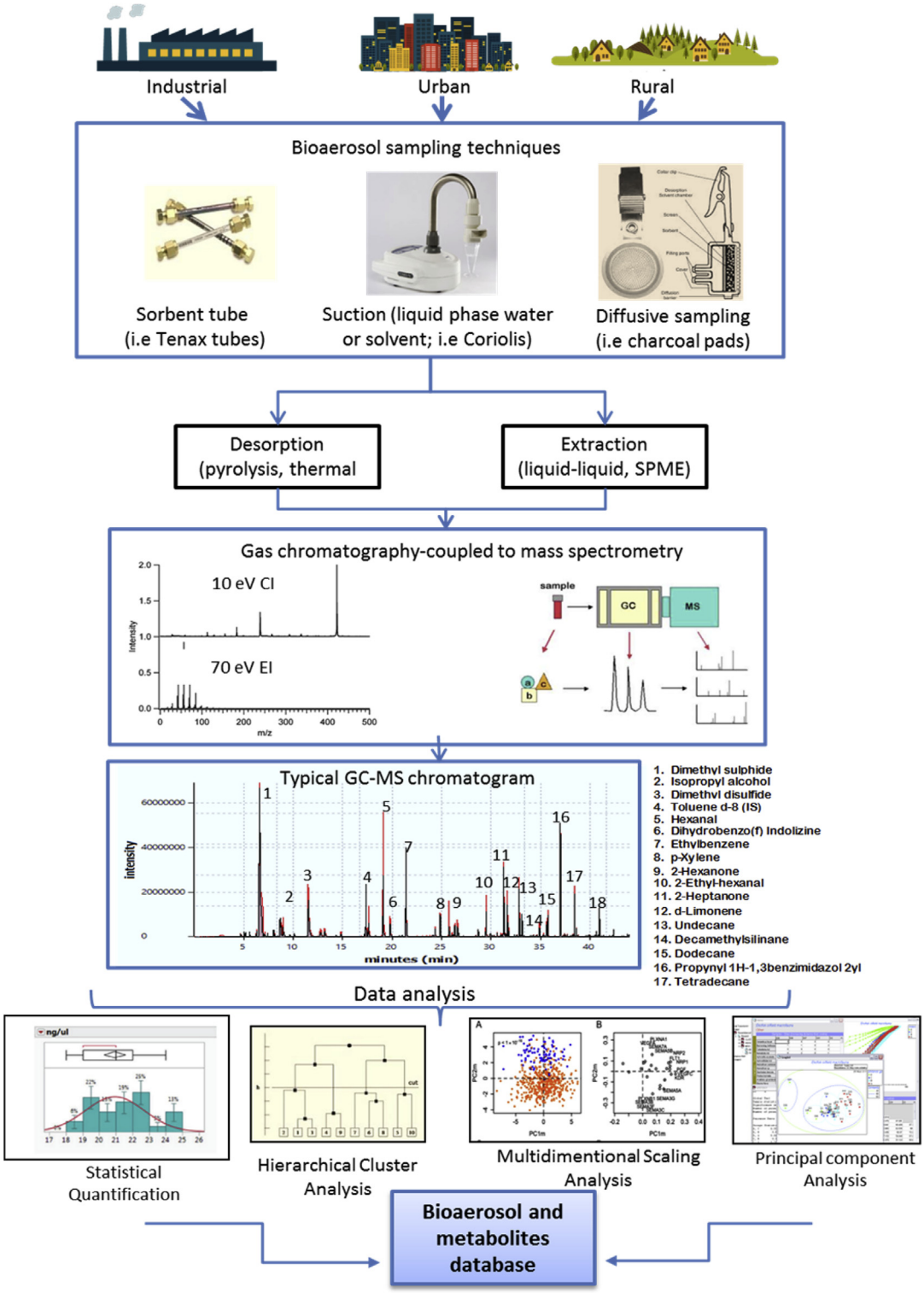

Fig. 1. Schematic representation of different sampling techniques depending on the downstream analysis required.

instance, the recoveries for less volatile and reactive MVOCs such as amines, phenols, aldehydes and unsaturated hydrocarbons are poor [34,47]. To sample carbonyl compounds, TD coated with Sep-Pak® gels are the most efficient, but the recoveries of the rest of the MVOCs are not good [44,62]. TD tubes are directly desorbed into the GC–MS avoiding sample preparation [28,58]. This is a rapid technique which is very sensitive in environments where MVOCs levels are low ($\leq$250 pg L^{-1}) [42] and it also provides good MVOC recoveries and low bias for analysis [33].

2.1.4. Electronic nose

Electronic noses have been used to monitor MVOCs concentrations because they are portable and provide real time analysis [40]. These devices are formed by electronic chemical sensors combined

Table 2
Advantages and limitations of different sampling and analytical techniques.

		Sampling time	Sampling volume	Sample preparation	Sensitivity	Real time analysis	Portable	Suitable for MVOCs analysis	References
Sampling techniques	Activated charcoal pads	Long	High	Yes	Low	No	Yes	Volatile MVOCs only	[47,52]
	Impingers	Short	High	Yes	Low	No	Yes	No	[55,63,64]
	Tenax® Desorption tubes	Short	Low	No	High	No	Yes	Yes	[28,31,42,65]
	Activated charcoal Desorption tubes	Long	High	Yes	Low	No	Yes	Very volatile MVOCs only	[34,47]
	Sep-Pak cartridges	Short	High	Yes	Low	No	Yes	Carbonyl compounds only	[44]
	Electronic nose	Short	Low	Yes	Low	Yes	Yes	Limited	[40,62,66]
Analytical techniques	GC–MS	n.a.	n.a.	n.a.	High	No	No	Yes	[47,51,67]
	GC–HS–SPME	n.a.	n.a.	n.a.	High	No	No	Yes	[47,67–71]
	Liquid chromatography	n.a.	n.a.	n.a.	Low	No	No	No	[60,72]

n.a. = not applied.

with an information processing unit, pattern recognition software, and a reference library which qualitatively recognises volatile organic compounds from simple or complex odours [61,62]. The use of an electronic nose for fungal detection has potential in clinical and pathological diagnoses as well as in food safety industry [62]. However, for detection and identification of outdoor MVOCs for instance, the limit of detection is poor and the sensor can easily be activated and make false measurements. Apart from this, the samples need to be cleaned and pre-concentrated in order to remove interferences [40].

2.2. Analytical techniques for MVOCs fingerprinting

Due to the volatile character of the MVOCs, these are commonly analysed by gas chromatography coupled to mass spectrometry (GC–MS) [73]. The use of MS has already been reported for the analysis of MVOCs including the Bioaerosol Mass Spectrometer (BMAS) developed for the detection of microorganisms. GC–MS is very specific and has a very low limit of detection (pg m^{-3}) which allows both the identification and quantification, even at low concentration, of MVOCs from complex environmental matrices. However, a combination of both chemical impact (CI) and electron impact ionizations (EI) should be considered when analysing MVOCs, as several studies reported that some MVOCs are only identified when using a specific ionization source. For example, 2-pentanol is not detected by chemical impact (CI) ionization and 2-methyl-1-butanol is not detected by electron impact ionization (EI). Hence EI and CI should be used as complimentary mass spectrometry methods to study MVOCs in environmental matrices [47,67]. MVOCs from TD tubes are desorbed and directly connected to the GC–MS [48]. When MVOCs are collected on a charcoal pad they are solvent extracted although solvent extraction and heat produce VOC degradation products [47].

Headspace solid phase micro-extraction (HS-SPME) is the most widely used and established extraction method of MVOCs from liquid samples. MVOCs are extracted with a short fused silica fibre and desorbed at high temperatures-without the need of any solvent [67]. This is a robust technique which is very sensitive at trace levels (pg L^{-1} to ng L^{-1}) and it has a powerful separation capacity. In contrast, the extraction efficiency is limited and some MVOCs might not be detected [47,66]. HPLC techniques are less commonly used as they are only efficient for the analysis of carbonyl compounds but are not very suitable for volatile compounds [60].

2.3. MVOC separation, speciation and data mining

Future trends are moving towards the identification of microorganisms existing in air using chemometrics as this is a quicker and a cost effective approach compared to cell culturing or molecular analysis [24]. Chemometrics is the analysis of chemical data using mathematics and statistics to extract maximum chemical information from a sample by optimising process data analysis, signal processing and multivariate analysis [74]. Thus, firstly peak identification of the chromatogram is performed with a MS database (i.e., NIST, ADMIS, METLIN, mzCloud, etc). Peak deconvolution allows accurate mass spectra for the abundant and sometimes coeluting peaks from complex chromatograms that could otherwise be overlooked when scanning. Due to the large number and varying concentrations of MVOCs obtained from the analysis, multivariate analysis is required to recognize patterns from the different environmental samples [26,70]. Principal component analysis (PCA), HCA or Multidimensional scaling analysis (MDS) are performed with the *m/z* spectra. A wide range of statistical software (ADAPT, MATLAB, etc.) or chemometric software (ACD/MS Manager, Mass Profiler Professional, OpenChrom, SpectConnect) can be used [75]. Identifying species-specific MVOCs is not easy because not all MVOCs are specific to specific microbial species [76]. Vishwanath et al. [36] did not succeed in finding a correlation between MVOCs, VOCs and secondary metabolites from environmental samples due to the ambiguity of MVOCs and the lack of certified reference environmental samples reporting MVOCs levels. Terpenes and sesquiterpenes can either be from a microbial origin (MVOCs) or from chemicals (VOCs) (cosmetics, perfumes, fruits, cleaning products, wood etc) [77]. Methylfuranes can also be emitted from tobacco smoke by the pyrolysis of tobacco components [78] and other MVOCs have been related to VOCs emitted from building materials [40].

There are generic MVOCs (Table 3) which can be uniquely related to microbial activity such as 2-hexanone and 3-methyl-1-butanol. However, they are emitted by all microorganisms (fungi and bacteria) and therefore their detection cannot be attributed to any specific microbe. There are also other MVOCs like 3-octanol that are only emitted by fungi and compounds such as 3-methyl-2-butanol, Geosmin, borneol, 2-methyl-isorbenol and 2-isopropryl-3-methoxypyrazine that are solely emitted by bacteria (Table 3).

To date, only a limited number of MVOCs can be attributed to genus level and rarely to species level due to their complexity. In fact, the literature shows discrepancies about which MVOC is emitted by which microorganism. For example, one study reported 2-pentyl furan as specific MVOC from *A. fumigatus* [32] whereas other studies reported that it was also emitted by other *Aspergillus* sp. (*Aspergillus terreus*, *Aspergillus flavus* and *Aspergillus niger*) and other fungus genera (*Fugarium* spp and *Scedosporium apiospermum*) as well as by the bacteria *Streptococcus pneumonia* [29,80]. Initially 3-octanone was thought to be specific from *A. fumigatus* but other research groups found that it was also emitted by *A. flavus* [81]. Based on the critical review carried out in

Table 3
Microbial origin and chemical properties of most frequently reported MVOCs in the environment [24,38,42,76,79].

Chemical group	Compound	Microbial origin	Chemical formula	Molecular weight	Boiling point (°C) at 101.3 kPa	Vapour pressure (kPa at 25 °C)	[a]log K_{OW}
Alcohols	2-Methyl-1-propanol	Bacteria and fungi	$C_4H_{10}O$	74.12	108	1.33	0.65–0.83
	2-Methyl-1-butanol	Bacteria and fungi	$C_5H_{12}O$	88.15	128	0.416	1.29
	3-Methyl-1-butanol	Bacteria and fungi	$C_5H_{12}O$	88.15	130.5	0.316	1.16
	3-Methyl-2-butanol	Bacteria	$C_5H_{12}O$	88.15	111.5	1.22	1.28
	3-Octanol	Fungi	$C_8H_{18}O$	130.23	169	0.068	2.73
	1-Octen-3-ol	Bacteria and fungi	$C_8H_{16}O$	128.21	180	0.071	2.6
	2-Octen-1-ol	Bacteria and fungi	$C_8H_{16}O$	128.21	195.8 ± 8.0	0.014	2.59
	2-Pentanol	Bacteria and fungi	$C_5H_{12}O$	88.15	119.0–119.3	0.815	1.19
	Methanol	Bacteria and fungi	CH_4O	32.04	64.7	32	−0.5
Ethers	2-Methylfuran	Bacteria and fungi	C5H60	82.1	65	23.48	1.85
	3-Methylfuran	Bacteria and fungi	C_5H_6O	82.1	65–66	21.46	1.91
Ketones	2-heptanone	Bacteria and fungi	$C_7H_{14}O$	114.19	150.6–151.5	0.213–0.28	2.03
	2-hexanone	Bacteria and fungi	$C_6H_{12}O$	100.16	126–128	1.47,0.36	1.38
	3-Octanone	Bacteria and fungi	$C_8H_{16}O$	128.21	157–162	0.267	2.22
Terpenes	Geosmin	Bacteria	$C_{12}H_{22}O$	182.31	252.4 ± 8.0	0.00041	3.57
	Borneol	Bacteria	$C_{10}H_{18}O$	154.25	213	0.009	2.30
	2-methylisorbenol	Bacteria	$C_{11}H_{20}O$	168.28	208.7 ± 8.0	0.0065	3.31
	β-Caryophyllene	Bacteria	$C_{15}H_{24}$	204.35	254	NA	NA
	α-Pinene	Bacteria and fungi	$C_{10}H_{16}$	136.23	155	0.4	2.8
	Camphene	Fungi	$C_{10}H_{16}$	136.23	159	NA	3.3
	Camphor	Bacteria and fungi	$C_{10}H_{16}O$	152.23	209	0.53	2.2
Sulphur and nitrogen compounds	Dimethylsulfide	Bacteria and fungi	C_2H_6S	62.134	188.8	53.7	0.977
	Dimethyl disulfide	Bacteria and fungi	$C_2H_6S_2$	94.19	109.8	3.83	1.77
	2-Isopropyl-3-methoxypyrazine	Bacteria	$C_8H_{12}N_2O$	152.2	210.8 ± 30.0	0.036	2.37
Aldehydes	Acetaldehyde	Bacteria and fungi	C_2H_4O	44.05	20.2	101	−0.3
	Furfural	Bacteria and fungi	$C_5H_4O_2$	96.08	162	0.15	0.41
Acids	Butanoic acid	Bacteria and fungi	$C_4H_8O_2$	88.10	163.75	0.74	0.8
	Propanoic acid	Bacteria	$C_3H_6O_2$	74.07	141.15	0.47	0.3

[a] log K_{OW} = Octanol–Water partition coefficient.

this study and the MVOCs database developed by Lemfack et al. [24], we have found a set of MVOCs which are less commonly reported but are more specific (at genus or species level) (see Table 4). Previous research with *in vitro* cultures have shown the possibility to differentiate fungal species based on MVOCs emissions; this is called chemotyping or chemotaxonomy [55,82–84]. Apart from identifying these MVOCs in air, significant developments are required towards the analytical approaches to advance metabolite annotation in different ambient environments. A sensitive and portable GC–MS unit coupled with SPME or a more improved electronic nose that MCOCs at trace levels would be crucial in order to perform real time analysis as current analytical techniques are lab based and cannot provide us with immediate results. Furthermore, species-specific MVOCs could serve as markers for the rapid identification of microbes.

3. Conclusions and future perspectives

Bioaerosols have been studied for over 30 years, but there is still a need to expand our understanding and ability to characterise, identify and quantify these and their metabolites from different ambient environments. Characterisation of MVOCs has a potential to be used as a tool to elucidate atmospheric bioaerosols. Hence there is a need to develop a rapid, reliable and replicable procedure for sample collection and analysis of microbial VOCs. Due to the nature of the outdoor environment as an open system, outdoor MVOCs concentrations are lower than those indoors and therefore powerful sample capture and analytical techniques are required. Analytical techniques have become more sensitive and nowadays allow us to detect compounds at trace levels. In addition, analytical techniques are quicker and more cost effective than the conventional microbial and molecular approaches. Up to now, there is no technique that allows the detection of MVOCs at environmental levels in real time. Therefore, future trends should move towards the chemical characterisation of bioaerosols looking at MVOCs. TD sampling tubes coupled with GC–MS analysis seems to be the most sensitive and robust technique, being also the most extensively used one. Impingement techniques with a Coriolis®μ is also a promising device as it is portable, easy to use and without microbial loss, despite there being issues with sampling efficiency. Liquid samples from impingers are directly analysed in GC–MS coupled with a headspace (HS) autosampler without the need of sample preparation. Compared to TD-GCMS, HS autosampler is not as sensitive and a SPME fibre needs to be added in the injector to concentrate the analytes before the injection into the GC–MS. Unfortunately there does not seem to be a unique analytical technique suitable for all the groups of MVOCs and the technique that covers the maximum range of compounds offering a robust, reproducible and sensitive analysis should be chosen. Depending on the sample device used, different sample injection will be needed (SPME, solvent injection, TD-GCMS). Apart from these, at the moment there is a lack of techniques available for real time on site detection of MVOCs as most of the analytical techniques allowing MVOC detection at low concentrations are lab based. A development of a more sensitive and portable GC–MS unit coupled with SPME or an electronic nose would be required for a rapid detection of MVOCs. The combination of the chemical characterisation of bioaerosols with speciation analysis are identified as the next step for the identification of species specific MVOCs. Future perspectives should move towards the development of a rapid analysis and evaluation of ambient air creating a standardised database of the most abundant MVOCs found in a range of environments (industrial, urban and rural). This database should contain specific MVOCs for each of the most relevant microbes in air to be able to immediately identify the microbial pathogens without the need of culturing them or applying molecular techniques.

Table 4
Possible specific MVOCs and its physicochemical properties.

Microorganism	References	Specific MVOCs	Molecular formula	Molecular weight (g mol^{-1})
Aspergillus flavus	[85]	cis2-octen-1-ol	$C_8H_{16}O$	128.21
Aspergillus fumigatus	[66,85]	2,4-Pentadione (Acetylacetone)	$C_5H_8O_2$	100.12
		3-Methyl-1,3-pentandione	$CH_3COCH(CH_3)COCH_3$	114.14
		p-Mentha-6,8-dien-2-ol acetate	$C_{12}H_{18}O_2$	194.27
Aspergillus versicolor	[47,85]	Trimethylnonanoic acid methylester	$C_{14}H_{28}O_2$	228.37
		1-(3-Methylphenyl)-ethanone	$C_9H_{10}O$	134.18
Aspergillus candidus	[85,86]	3-Cyclohepten-1-one isomer	$C_{18}H_{32}O_2$	280.45
Emericella nidulans	[86]	beta-Fenchyl alcohol	$C_{10}H_{18}O$	154.25
		2-Methyl-butanoic acid methyl ester	$C_7H_{14}O_2$	130.18
		4,4-Dimethyl-pentenoic acid methyl ester	n/a	n/a
Penicillium clavigerum	[85,86]	Bicyclooctan-2-one	$C_8H_{12}O$	124.18
Penicillium crustosum	[85,86]	2-Ethyl-5-methyl-furan	$C_7H_{10}O$	110.15
		4-Ethylbutan-4-olide ((S)-gamma-hexalactone)	$C_6H_{10}O_2$	114.14
		Isopropylfuran	$C_7H_{10}O$	110.15
Penicillium cyclopium	[85,86]	2-Methyl-2-bornene isomer	n/a	n/a
		delta-2-Dodecanol	n/a	n/a
		4-Methyl-2-(3-methyl-2-butenyl)-furan	$C_{10}H_{14}O$	150.22
Penicillium roqueforti	[66]	beta-patchoulene-isomer	$C_{15}H_{24}$	204.35
		beta-elemene-isomer	$C_{15}H_{24}$	204.36
		(1,1-dimethylethyl)-2-methylphenol	n/a	n/a
		Butanoic acid, 2-methyl-2-methylpropyl ester	$C_8H_{16}O_2$	144.21
		alpha-selinene	$C_{15}H_{24}$	204.35
		1-methyl-4-(1-methylethyl) benzene (p-Cymene)	$C_{10}H_{14}$	134.22
		Propanoic acid 2-methyl-2-methylpropyl ester (or Propanoic acid, 2-methyl-3-methylbutyl ester or Isobutyric acid)	$C_8H_{16}O_2$	144.21
		alpha-chamigrene	$C_{15}H_{24}$	204.35
Paecilomy cesvariotii	[4]	3,5,7-Trimethyl-2E,4E,8E-decatetraene	$C_{13}H_{20}$	176.30
		2-Methyl-2,4-hexadiene	C_7H_{12}	96.17
		delta-4-Carene	$C_{10}H_{16}$	136.23
Trichodema pseudokoningii	[4]	2-Methyl-pentane	C_6H_{14}	86.18
Muscodor crispans	[4]	Hexane, 2,3-dimethyl-	C_8H_{18}	114.23
		Formamide, N-(1-methylpropyl)	$C_7H_{15}NO_2$	145.20
		Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)	$C_{14}H_{24}$	192.34
Arthrobacter globiformis	[4]	2-Phenylethylamine	$C_8H_{11}N$	121.18
Mycobacterium	[4]	5-Methylhexan-3-ol	$C_7H_{16}O$	116.20
		7-Methyloctan-3-one	$C_9H_{18}O$	142.24
		5-Methyl-4-hexen-3-one	$C_7H_{12}O$	112.17
		Cyanoisoquinoline	$C_{10}H6N_2$	154.17
Bacillus spp.	[4]	(2R,3R)-Butane-2,3-diol	$C_4H_{10}O_2$	90.12
Geobacillus stearothermophillus	[4]	Dimethyl ditelluride	C_2H_6Te	157.67
		Methanetellurol	CH_4Te	143.64
		dimethylselenodisulfide	n/a	173.15
		dimethyltellurenalsulfide	n/a	189.73
Paenibacillus polymyxa	[4]	2-(2-Methylpropyl)pyrazine	$C_9H_{14}N_2O$	166.22
		2,6-Diisobutylpyrazine	$C_{12}H_{20}N_2$	192.30
		2-Methyl-5-isobutylpyrazine	$C_9H_{14}N_2$	150.22
Stapahylococcus aureus	[4]	2,3,4,5- tetrahydropyridazine	$C_4H_8N_2$	84.12
		4-methylhexanoic acid	$CH_3CH_2CH(CH_3)CH_2CH_2COOH$	130.18
		Butyl butanoate (butyl butyrate)	$C_8H_{16}O_2$	144.21
Pseudomonas sp.	[87]	2,4-Diacetylphloroglucinol	$C_{10}H_{10}O_5$	210.18
Pseudomonas trivialis	[85]	Undecadiene	$C_{11}H_{20}$	152.28
		Benzyloxybenzonitrile	$C_{14}H_{11}NO$	209.25
Escherichia coli	[85]	Pentylcyclopropane	C_8H_{16}	112.21
Acinetobacter calcoaceticus	[85]	Sulfoacetaldehyde	$C_2H_4O_4S$	124.12
Klebsiella sp.	[85]	Pentylbutanoate (or pentyl butyrate)	$C_9H_{18}O_2$	158.24
Streptomyces citreus	[85]	Dihydroagarofuran (sesquiterpenoid)	$C_{15}H_{26}O$	222.37
		Bicyclogermacrene	$C_{15}H_{24}$	204.35
		betabourbonene	$C_{15}H_{24}$	204.35
		delta-elemene	$C_{15}H_{24}$	204.36
Alternaria alternata	[85]	6-Methylheptanol	$C_8H_{18}O$	130.23
Rhizopus stolonifer	[85]	1-Octene	C_8H_{16}	112.24
		3-Methyl-3-buten-1-ol	$CH_2{=}C(CH_3)CH_2CH_2OH$	86.13

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trac.2016.10.010.

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